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Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Molecules interacting with apoptin

(57) The invention relates to activation of apoptosis by means of interference of Hou-like and/or IFP35-like compounds.

Also the invention relates to anti-tumor therapies with compounds, which negatively interfere with Hou-like and/or IFP35-like compounds leading to induction of apoptosis, resulting in the elimination of tumor cells.

Also the invention relates to therapies for diseases related to aberrant apoptosis induction, such as auto-immune disease.

Also the invention describes the diagnosis of cells, which are susceptible to apoptin- or apoptin-like induced apoptosis.

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new treatments and diagnosis for diseases related with aberrancies in the apoptotic process, such as cancer and auto-immune diseases.

[0013] Proteins found associating with apoptin include members of the family of Nmi/Hou-like and IFP-like proteins.

[0014] Thus the invention provides a recombinant and/or isolated nucleic acid molecule encoding at least a functional part of a member of the family of Nmi-like proteins or at least a functional part of a member of the family of Hou-like proteins or at least a functional part of a member of the family of IFP35-like proteins for use in the induction of apoptosis in a population of cells related to a pathological condition.

[0015] As explained herein the expression of Hou is connected to oncogenes and has been found to be high in certain transformed cells. These are typically the cells that can be induced to go into apoptosis by apoptotic agents such as apoptin. Typically providing a cell with Hou-like activity will therefor increase the chance of inducing apoptosis in such a cell. IFP35-like proteins are involved in transporting apoptotic substances to the nucleus of cells. Under influence of for instance interferons these proteins localize in the nucleus. Therefor IFP-like activity is used to get apoptin-like activity into the nucleus, which is important for the induction of apoptosis, for instance through Hou-like proteins. The Hou-like activity or Nmi-like activity is defined herein as any molecule capable of exerting the sanme or a similar function as the original Hou-like (Nmi-like) protein. The same definition goes for IFP-activity. Typically such a molecule can be encoded by a nucleic acid molecule which comprises at least a functional and specific part of the sequence of figure 1, 2, 4 or 5 or encoding an amino sequence of figure 6 or a sequence at least 60, preferably 70, preferably 90 % homologous with said functional and specific sequence or comprising a sequence hybridizing to any of the aforegoing sequences under stringent conditions. In order to be able to express the Hou-like activity and/or the IFP-like activity it is preferred to have an expression vector encoding said activity. Expression vectors are nucleic acid molecules which can be brought into cells, or transfect cells themselves and which have the machinery (together with the machinery of the host cell) to express proteins encoded on the expression vector when present in a cell.

[0016] It is preferred that cells which are provided, according to the invention, with Hou-like activity and/or IFP-like activity, are also provided with apoptosis inducing activity, preferably apoptin-like activity, which is defined along the same lines as Hou-like activity. In order to get the activity into the cells in which apoptosis has to be induced it is possible and preferred to use a gene delivery vehicle. A gene delivery vehicle is a means to transport a nucleic acid molecule capable of expressing the wanted activity in a host cell into said host cell. Gene delivery vehicles are known in the art. They include for instance recombinant viruses such as adenoviruses and retroviruses, but also non-viral vehicles such as polymers and liposomes have been suggested. Methods of targeting gene delivery vehicles to target cells are also known in the art and need not be elaborated herein. The invention also provides the newly identified molecules themselves, both the nucleic acid molecules (meaning DNA coding and/or non coding strands as well as RNA) and the proteinaceous molecules (peptides, polypeptides, glycoproteins and associations between prtoeins and RNA's and the like). Based on the given sequences other familymembers of the Hou/Nmi and IFP families will be identified having the same or similar function. Typically such molecules will have high homology to the sequences given herein.

[0017] For nucleic acid molecules the homology is exppected to be at least 60, preferably 70, more preferably 80%. therewith.

[0018] These nucleic acid molecules can of course again be incorporated into expression vectors as mentioned hereinbefore. Preferably these expression vectors also encode apoptotic activity, preferably apoptin or a functional fragment and/or equivalent thereof.

[0019] These expression vectors can again be made into gene delivery vehicles.

[0020] The invention also provides the recombinant or isolated proteinaceous substance comprising at least a functional part of a member of the family of Nmi/Hou-like proteins or at least a functional part of a member of the family of Hou-like proteins for use in the induction of apoptosis in a population of cells related to a pathological condition and an Nmi/Hou-like proteinaceous substance having at least a functional and/or specific part of the sequence of figure 3 or being encoded by a functional and/or specific part of the sequence of figure 1 or figure 2 or being at least 60, preferably 70, preferably 80% homologous to at least a functional and/or specific part of the sequence of figure 3 or being at least 60, preferably 70, preferably 80% homologous to a protein encoded by at least a functional and/or specific part of the sequence of figure 1 or figure 2 and an IFP35-like proteinaceous substance having at least a functional and/or specific part of the sequence of figure 6 or 7 or being encoded by a functional and/or specific part of the sequence of figure 4 or figure 5 or being at least 60, preferably 70, preferably 80% homologous to at least a functional and/or specific part of the sequence of figure 6 or 7 or being at least 60, preferably 70, preferably 80% homologous to a protein encoded by at least a functional and/or specific part of the sequence of figure 4 or figure 5.

[0021] A functional part in this document means having the same or similar activity (although the amount of activity may differ) A specific part herein means a part of sufficient size to be specific for the protein or nucleic acid or to be of sufficient size to distinguish the protein from another protein immunologically. The proteins disclosed herein can for instance also be used to identify further components of the apoptotic pathway.

[0022] The reason for bringing IFP-like activity and/or Hou-like activity together with apoptotic activity is of course to induce aberrant cells to go into apoptosis. Thus the invention also provides a method for inducing apoptosis in cells

GAL4-activation domain-tagged cDNA library

[0043] The expression vector pACT, containing the cDNAs from Epstein-Barr-virus-transformed human B cells fused t the GAL4 transcriptional activation domain, was used for detecting apoptin-associating proteins. The pACT c-DNA library is derived from the lambda-ACT cDNA library, as described by Durfee et al. 1993.

Bacterial and Yeast strains

[0044] The E.coli strain JM109 was the transformation recipient for the plasmid pGBT9 and pGBT-VP3. The bacterial strain electromax/DH10B was used for the transformation needed for the recovery the apoptin-associating pACT-cDNAs, and was obtained from GIBCO-BRL, USA.
[0045] The yeast strain Y190 was used for screening the cDNA library, and all other transformations which are part of the used yeast-two-hybrid system.

Media

[0046] For drug selections Luria Broth (LB) plates for E.coli were supplemented with ampicillin (50 microgram per ml). Yeast YPD and SC media were prepared as described by Rose et al. (1990).

Transformation of competent yeast strain Y190 with plasmids pGBT-VP3 and pACT-cDNA and screening for beta-galactosidase activity.

[0047] The yeast strain Y190 was made competent and transformed according to the methods described by Klebe et al. (Klebe et al., 1983). The yeast cells were first transformed with pGBT-VP3 and subsequently transformed with pACT-cDNA, and these transformed yeast cells were grown on histidine-minus plates, also lacking leucine and tryptophan.
[0048] Hybond-N filters were layed on yeast colonies , which were histidine-positive and allowed to wet completely. The filters were lifted and submerged in liquid nitrogen to permeabilize the yeast cells. The filters were thawed and layed with the colony side up on Whattman 3MM paper in a petridish with Z-buffer (Per liter: 16.1 gr $Na_2HPO_4.7H_2O$, 5.5 gr $NaH_2PO_4.H_2O$, 0.75 gr KCl and 0,246 gr $MgSO_4.7H_2O$, pH 7.0) containing 0.27% beta-mercapto-ethanol and 1 mg/ml X-gal. The filters were incubated for at least 15 minutes or during night.

Recovery of plasmids from yeast

[0049] Total DNA from yeast cells, which were histidine- and beta-galactosidase-positive, was prepared by using the glusulase-alkaline lysis method as described by Hoffman and Winston (1987) and used to transform Electromax/DH10B bacteria via electroporation using a Bio-Rad GenePulser according the manufacturer's specifications.
[0050] Transformants were plated on LB media containing ampicillin.

Isolation of apoptin-associating pACT clones

[0051] By means of colony-filter assay the colonies were lysed and hybridized to a radioactive-labeled 17-mer oligomer, which is specific for pACT (see also section Sequence analysis).
[0052] Plasmid DNA was isolated from the pACT-clones, and by means of XhoI digestion analysed for the presence of a cDNA insert.

Sequence analysis

[0053] The subclones containing the sequences encoding apoptin-associating proteins were sequenced using dideoxy NTPs according to the Sanger method which was performed by Eurogentec, Nederland BV (Maastricht, The Netherlands). The used sequencing primer was a pACT-specific 17-mer comprising of the DNA-sequence 5'-TACCACTACAATGGATG-3'.
[0054] The sequences of the apoptin-associating proteins were compared with known gene sequences from the EMBL/Genbank.

Results and discussion

[0055] Apoptin induces specifically apoptosis in transformed cells, such as cell lines derived from human tumors. To identify the essential compounds in this cell-transformation-specific and/or tumor-specific apoptosis pathway, a yeast

Nmi, or Hou will be interchangeably used.

[0070] In this respect, the pattern of Nmi expression is interesting, since it is expressed at low levels in normal tissues, in contrast to its high levels of expression in transformed cell lines. Among eight cancer lines tested, highest levels were observed in four leukemia cell lines (Ba and Zervos, 1996).

[0071] In leukemias, a high expression of C-myc correlates with a high level of Nmi (HL-60, K562 and MOLT-4). The Nmi gene is located on chromosome 22, which is also involved in the t (9;22) translocation leading to the Bcr-Abl fusion protein, as seen in some leukemias (Rabbits, 1991, Sawyers and Deny, 1994).

[0072] Using a yeast genetic screen, Nmi was identified as a protein that binds to N-myc and C-myc. Myc proteins are important in the regulation of cell proliferation and differentiation. Together with ras or raf, myc can transform primary cells in culture. Nmi/Hou-like proteins will up-regulate the activity of Myc proteins via binding to them.

[0073] Up-regulation of Myc proteins has been described for Burkitt lymphomas, neuroblastomas and small cell lung carcinomas. Myc proteins contain a basic region, a helix-loop-helix (HLH) and a leucine zipper (Zip), and form homo-or heterodimers that can bind to specific DNA sequences and regulate transcription. Myc also forms heterodimers with Max. Myc/Max heterodimers activate transcription, whereas Max homodimers repress transcription, thus antagonizing Myc's function (Evan and Littlewood, 1993).

[0074] Nmi was found to interact with N-myc, c-myc, Max, Mxi1 and other transcription factors that have HLH and/or Zip motifs. Interaction with N-myc and C-myc was confirmed by co-precipitation experiments (Bao and Zervos, 1996).

Induction of apoptosis through interference with the function of Nmi/Hou-like proteins.

[0075] Our results indicate that apoptin can change the Nmi/Hou-like-mediated proliferation (transformation/tumor-formation) activity into a Nmi/Hou-like-mediated apoptotic activity. Remarkably, this Nmi/Hou-like-mediated apoptotic activity will be specific for transformed/tumor cells, due to the very high level of Nmi/Hou in transformed cells in combination with over-expression of (proto-)oncogenes, such as Myc.

[0076] By means of transient transfection assays, it was shown that over-expression of the determined Hou-like protein (see Fig. 3) and apoptin did result in induction of apoptosis in normal VH10-, VH25-fibroblasts. In contrast to normal fibroblasts which over-expressed only apoptin. This result indicates that Hou-like proteins are an important factor in (apoptin-induced) apoptosis.

[0077] The presented data imply that interference with the function of Nmi/Hou-like proteins resulting in apoptosis can be used as a specific anti-tumor therapy, or therapies of related diseases, such as auto-immune diseases.

Characteristics of the apoptin-associating protein IFP35

[0078] The other apoptin-associating protein is IFP35, which is an interferon(IFN)-induced leucine zipper protein of 282 a.a., and has an apparent molecular mass of 35 kD. It was isolated by differential screening from HeLa cells that had been treated with IFN-γ (Bange et al., 1994).

[0079] IFP35 mRNA could be induced by IFN-γ in different human cell types, including fibroblasts, macrophages, and epithelial cells. It has a leucine zipper motif at the N-terminus, but it lacks an adjacent basic domain required for DNA binding. It has been suggested that these types of proteins negatively regulate bZIP transcription factors by forming non-functional heterodimers. IFP35 was shown to form homodimers (Bange et al., 1994).

Induction of apoptosis by interference of IFP35 in combination with Hou/Nmi-like proteins.

[0080] IFP35 is found in the cell nucleus, after interferon treatment and is expressed in a wide variety of cell types including fibroblasts, macrophages and epithelial cells (Bange et al., 1994).

[0081] In general, virus infections trigger interferon production. It is likely that a CAV infection and/or expression of apoptin will result in interferon up-regulation, which might result in the translocation of IFP35 or IFP35-like proteins into the nucleus. IFP35 will transport apoptin also to the nucleus, due to its association.

[0082] It seems likely that if apoptin is transported into the nucleus by IFP35 it will be able to associate with the IFP35-homologous region within Hou/Nmi-like proteins. This association will cause an aberrant regulation of Hou/Nmi-regulated genes, such as the oncogene Myc. Subsequently, the cells over-expressing Nmi/Hou-like proteins and oncogenes, such as Myc will undergo apoptosis.

[0083] Experimental evidence for IFP35 as an essential factor in (apoptin) apoptosis induction was derived from the following experiments. Normal VH10 cells over-expressing Hou/Nmi, IFP35 and apoptin underwent faster apoptosis than normal VH10 cells expressing Hou/Nmi and apoptin.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(C) CITY: Leiden
(D) STATE: Zuid-Holland
(E) COUNTRY: the Netherlands
(F) POSTAL CODE (ZIP): 2333 AL

(ii) TITLE OF INVENTION: Novel molecules involved in apoptotic pathways.

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 97203781.6

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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(2) INFORMATION FOR SEQ ID NO: 2:

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(A) LENGTH: 658 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:89..716
(D) OTHER INFORMATION:/label= N
/note= ""N" stands for unknown."

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(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

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(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
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(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(B) TYPE: nucleic acid
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(D) TOPOLOGY: unknown

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(iii) HYPOTHETICAL: NO

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(D) OTHER INFORMATION:/label= N
/note= ""N" stands for unknown."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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AGCAAGTCCC 420

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GTCGCATCCA 480

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(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
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(iii) HYPOTHETICAL: NO

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        35                  40                  45

Pro Leu Val Phe Arg Gly His Thr Gln Gln Asp Pro Glu Val Pro Lys
    50                  55                  60

Ser Leu Val Ser Asn Leu Arg Ile His Cys Pro Leu Leu Ala Gly Ser
65                  70                  75                  80

Ala Leu Ile Thr Phe Asp Asp Pro Lys Val Ala Glu Gln Val Leu Gln
                85                  90                  95

Gln Lys Glu His Thr Ile Asn Met Glu Glu Cys Arg Leu Arg Val Gln
            100                 105                 110

Val Gln Pro Leu Glu Leu Pro Met Val Thr Thr Ile Gln Val Ser Ser
        115                 120                 125

Gln Leu Ser Gly Arg Arg Val Leu Val Thr Gly Phe Pro Ala Ser Leu
    130                 135                 140

Arg Leu Ser Glu Glu Glu Leu Leu Asp Lys Leu Glu Ile Phe Phe Gly
145                 150                 155                 160

Lys Thr Arg Asn Gly Gly Gly Asp Val Asp Val Arg Glu Leu Leu Pro
```

20 25 30

His Thr Ile Asn Met Glu Glu Cys Arg Leu Arg Val Gln Val Gln Pro
35 40 45

Leu Glu Leu Pro Met Val Thr Thr Ile Gln Val Met Val Ser Ser Xaa
50 55 60

Leu Ser Gly Arg Arg Val Leu Val Thr Gly Phe Pro Ala Ser Leu Arg
65 70 75 80

Leu Xaa Glu Glu Glu Leu Leu Asp Lys Leu Asp Leu Leu Trp Gln Xaa
85 90 95

Xaa Glu Arg Xaa Trp Arg Cys Gly Arg Ser Gly Ala Thr Ala Arg Glu
100 105 110

Cys His Ala Gly Val Cys Tyr Gly Trp Ser Gly Ser Ala Ser Val Pro
115 120 125

Asn Arg Pro Val His Lys Cys His Trp Val Gly Ser Lys Ser Leu Glu
130 135 140

Ser Leu Arg Met Xaa Xaa Arg Ser Glu Cys Xaa Val Ala Ser Asn Ser
145 150 155 160

Ser Leu Xaa Tyr Trp Cys Ser Xaa Ser Xaa Leu Gly Leu Ala Pro Xaa
165 170 175

Xaa Met Xaa Ser Gly Arg Phe Asn Xaa Xaa Ser Pro Xaa Xaa Xaa Xaa
180 185 190

Gly Lys Xaa Xaa Pro Xaa Xaa Ser Xaa Xaa Xaa Xaa Ser Xaa Ala
195 200 205

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 647 amino acids
(B) TYPE: amino acid

Asp Leu Ser Leu Lys Ile Pro Glu Ile Ser Ile Gln Asp Met Thr Ala
165 170 175

Gln Val Thr Ser Pro Ser Gly Lys Thr His Glu Ala Glu Ile Val Glu
180 185 190

Gly Glu Asn His Thr Tyr Cys Ile Arg Phe Val Pro Ala Glu Met Gly
195 200 205

Thr His Thr Val Ser Val Lys Tyr Lys Gly Gln His Val Pro Gly Ser
210 215 220

Pro Phe Gln Phe Thr Val Gly Pro Leu Gly Glu Gly Gly Ala His Lys
225 230 235 240

Val Arg Ala Gly Gly Pro Gly Leu Glu Arg Ala Glu Ala Gly Val Pro
245 250 255

Ala Glu Phe Ser Ile Trp Thr Arg Glu Ala Gly Ala Gly Gly Leu Ala
260 265 270

Ile Ala Val Glu Gly Pro Ser Lys Ala Glu Ile Ser Phe Glu Asp Arg
275 280 285

Lys Asp Gly Ser Cys Gly Val Ala Tyr Val Val Gln Glu Pro Gly Asp
290 295 300

Tyr Glu Val Ser Val Lys Phe Asn Glu Glu His Ile Pro Asp Ser Pro
305 310 315 320

Phe Val Val Pro Val Ala Ser Pro Ser Gly Asp Ala Arg Arg Leu Thr
325 330 335

Val Ser Ser Leu Gln Glu Ser Gly Leu Lys Val Asn Gln Pro Ala Ser
340 345 350

Phe Ala Val Ser Leu Asn Gly Ala Lys Gly Ala Ile Asp Ala Lys Val

Val Ala Lys Gly Leu Gly Leu Ser Lys Ala Tyr Val Gly Gln Lys Ser
565 570 575

Ser Phe Thr Val Asp Cys Ser Lys Ala Gly Asn Asn Met Leu Leu Val
580 585 590

Gly Val His Gly Pro Arg Thr Pro Cys Glu Glu Ile Leu Val Lys His
595 600 605

Val Gly Ser Arg Leu Tyr Ser Val Ser Tyr Leu Leu Lys Asp Lys Gly
610 615 620

Glu Tyr Thr Leu Val Val Lys Trp Gly His Glu His Ile Pro Gly Ser
625 630 635 640

Pro Tyr Arg Val Val Val Pro
645

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 213 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

His Glu Gly Arg Gly Val Thr Gly Asn Pro Ala Glu Phe Val Val Asn
1 5 10 15

Thr Ser Asn Ala Gly Ala Gly Ala Leu Ser Val Thr Ile Asp Gly Pro
20 25 30

Ser Lys Val Lys Met Asp Cys Gln Glu Cys Pro Glu Gly Tyr Arg Val
35 40 45

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His Glu Gly Arg Pro Thr Glu Pro Gly Asn Tyr Ile Ile Asn Ile Lys
1 5 10 15

Phe Ala Asp Gln His Val Pro Gly Ser Pro Phe Ser Val Lys Val Thr
20 25 30

Gly Glu Gly Arg Val Lys Glu Ser Ile Thr Arg Arg Arg Arg Ala Pro
35 40 45

Ser Val Ala Asn Val Gly Ser His Cys Asp Leu Ser Leu Lys Ile Pro
50 55 60

Glu Ile Ser Ile Gln Asp Met Thr Ala Gln Val Thr Ser Pro Ser Gly
65 70 75 80

Lys Thr His Glu Ala Glu Ile Val Glu Gly Glu Asn His Thr Tyr Cys
85 90 95

Ile Arg Phe Val Pro Ala Glu Met Gly Thr His Thr Val Ser Val Lys
100 105 110

Tyr Lys Gly Gln His Val Pro Gly Ser Pro Phe Gln Phe Thr Val Gly
115 120 125

Pro Leu Gly Glu Gly Gly Ala His Xaa Val Arg Ala Gly Gly Pro Gly
130 135 140

Leu Xaa Lys Ser Ser Trp Ser Ala Ser Arg Ile Gln Tyr Leu Gly Pro
145 150 155 160

Gly Lys Leu Val Leu Glu Ala Trp Pro Leu Leu Ser Xaa Ala Pro Ala
165 170 175

induction of apoptosis in a population of cells related to a pathological condition.

14. An Nmi/Hou-like proteinaceous substance having at least a functional and/or specific part of the sequence of figure 3 or being encoded by a functional and/or specific part of the sequence of figure 1 or figure 2 or being at least 60, preferably 70, preferably 80% homologous to at least a functional and/or specific part of the sequence of figure 3 or being at least 60, preferably 70, preferably 80% homologous to a protein encoded by at least a functional and/or specific part of the sequence of figure 1 or figure 2.

15. A recombinant or isolated proteinaceous substance comprising at least a functional part of a member of the family of Nmi/Hou-like proteins or at least a functional part of a member of the family of Hou-like proteins for use in the induction of apoptosis in a population of cells related to a pathological condition.

16. An IFP35-like proteinaceous substance having at least a functional and/or specific part of the sequence of figure 6 or 7 or being encoded by a functional and/or specific part of the sequence of figure 4 or figure 5 or being at least 60, preferably 70, preferably 80% homologous to at least a functional and/or specific part of the sequence of figure 6 or 7 or being at least 60, preferably 70, preferably 80% homologous to a protein encoded by at least a functional and/or specific part of the sequence of figure 4 or figure 5.

17. A method for inducing apoptosis in cells comprising providing said cells with Nmi/Hou-like protein activity and/or IFP-35-like activity together with apoptin-like activity.

18. Use of apoptin to find proteinaceous substances associated with apoptosis.

CGGAGTTACAAGAGGCTACCAAAGAATTCCAGATTAAAGAGGATATTCCTGAAACAAAGATGAAA
TTCTTATCAGTTGAAACTCCTGANAATGACAGCCAGTTGTCAAATATCTCCTGTTCGTTTCAAGG
TGAGCTCGAAAGTTCCTTATGAGATACAAAAAGGACAATGCACTTATCACCTTTGAAAAAGGAAG
AAGTTGCTCAAAATGTGNGTAANGCATGAGTAAACATCATGTACAGATAATAAGATGTAAATCTG
GAGGTTACGGCCAAAGCCAAGTTCCATTAATATTCAAGGAGTCANGATTCCAGNGTTTATGCTAG
AANGTTTCTAAAAATGANAATCAATGGTTACTGGAAATTCCTGGACACATTGCGNTGAAAGATCA
AGATGACGAAGACAAACTAAGAAGCTGAGCTTTTCAAAAGTCCCGAAANATGGAAGAGCGGTAGA
GGGTGGNACCGCGTGNGANCTATGACAAGACAAGNCCGGGGAAGNTGCAGTCCATCACGTTTGTN
NGAAGATTGGANGTNGGCTGACCAANGAATTTTGAAAAAGGAGANGAATTACCCCTCTTTANGAG
TAANATCAAAACCCTGCCATAANAAGTTNACTGGTTTCNCCCATTACACAGNAN
TTACANNTTGANCAANANTANNCAGGATAATTTNCAGGGGAANAATCTNAAGNATGGCAAGNTGA
CTTCTGGACAANGGT

Figure 2

Hou c17/#2

AGCAGGTGCTGCAACAAAAGGAGCACACGATCAACATGGAGGAGTGCCGGCTGCGGGTGCAGGTC
CAGCCCTTGGAGCTGCCCATGGTCACCACCATCCAGGTGTCCAGCCAGTTGAGTGGCCGGAGGGT
GTTGGTCACTGGATTTCCTGCCAGCCTCAGGCTGAGTGAGGAGGAGCTGCTGGACAANCTANAGA
TCTTCTTTGGCAAGACTAGGAACGGAGGTGGCNATGTGGACNTTCGGGANCTACTGCCAGGGANT
GTCATGCTGGGGTTTGCTAGGGATGGAGTGGCTCANCGTCTGTGCCAAATCGGCCATTTCACAGT
GCCACTGGGTGGGCAGCANGTCCCTCTGAGAGTCTCTCCGTATGTGAATGGGGANATCCAGANGG
CTGANATCAGGTCNCAGCCANTTCCCCGCTCGGTACTGGTGCTCAACATTCCTGATATCTTGGAT
GGCCCGGAGCTGCATGACGTCCTGGANATCCACTTCCAGAANCCCACCCGCGGGGGCGGAGATGT
AAGACGCCCTGACAGTCGTACCCCAAGGACAACAGGGCCTAACAGTCTTCACCTCCTGAATCAAG
GCTANGGGCCTCCCCCTTCTCATCCTCCCCACCCCCCCCGCCAAAGGTTCTCAANACTGGGCCTG
GGCTTTNTG

Figure 4

IFP35 c14/#1

GGATCCACTGCCCTCTGCTTGCGGGCTCTGCTCTGATCACCTTTGATGACCCCAAAGTGGCTGAG
CAGGTGCTGCAACAAAAGGAGCACACGATCAACATGGAGGAGTGCCGGCTGCGGGTGCAGGTCCA
GCCCTTGGAGCTGCCCATGGTCACCACCATCCAGGTGATGGTGTCCAGCCANTTGAGTGGCCGGA
GGGTGTTGGTCACTGGATTTCCTGCCAGCCTCAGGCTGANTGAGGAGGAGCTGCTGGACAAGCTA
TGAGATCTTCTTTGGCAANACTANGAACGGANGTGGCGATGTGGACGTTCGGGAGCTACTGCCAG
GGAGTGTCATGCTGGGGTTTGCTACGGATGGAGTGGCTCAGCGTCTGTGCCAAATCGGCCAGTTC
ACAAGTGCCACTGGGTGGGCAGCAAGTCCCTCTGAGAGTCTCTCCGTATGTGANTGGNGAGATCA
GAATGCTGANATTAAGTCGCATCCAATTCCTCGCTCNGGTACTGGTGCTCANNATCCTGANATCT
TGGATTGGCCCCNGANTNCATGANATCTGGNAGATTCAATTNCANAAGTCCANCCNNCNGNGNCG
GGAAGTANANGCCCGANANTTCNTNNCNTANGGNCAGCANNGCCTG

Figure 6

IFP35 c51/#3

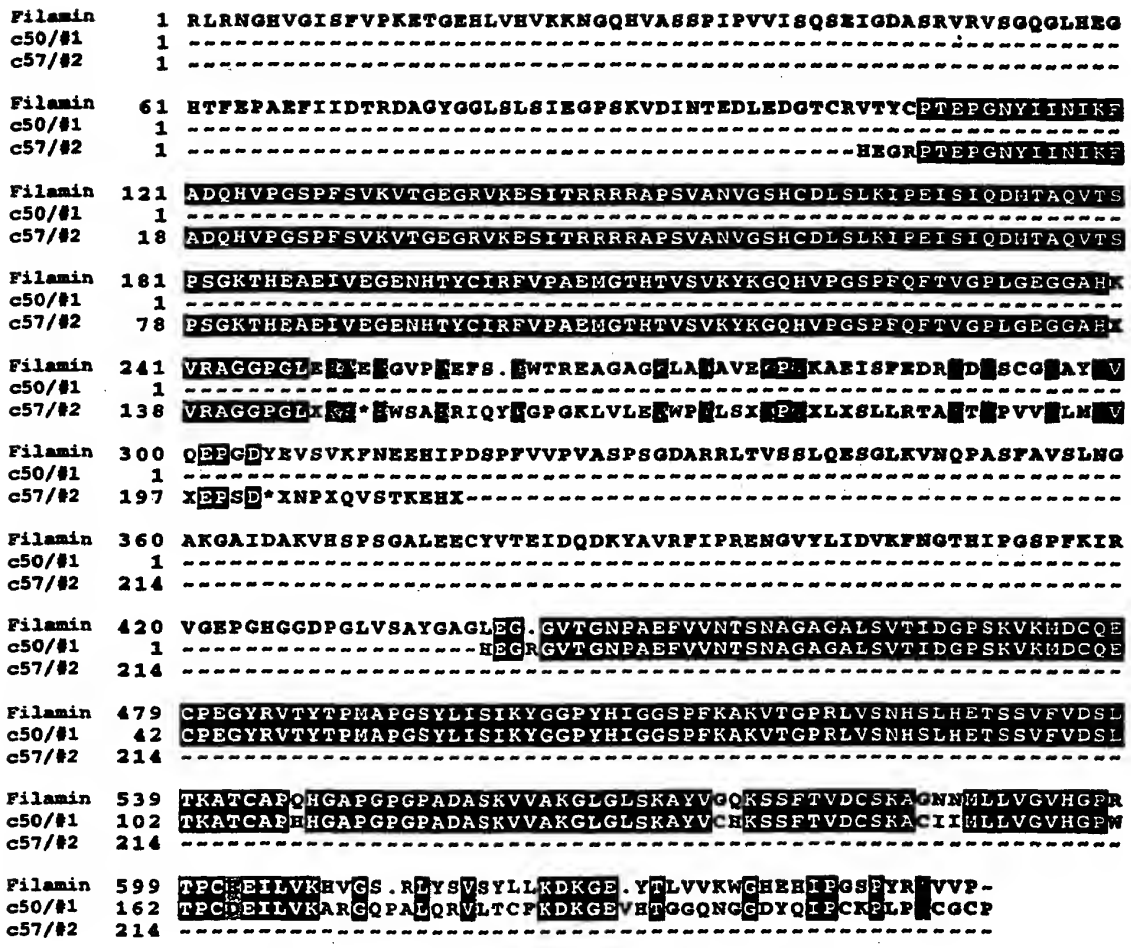

Figure 8

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 97 20 3781

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	ZHUANG S -M ET AL: "APOPTIN, A PROTEIN ENCODED BY CHICKEN ANEMIA VIRUS, INDUCES CELL DEATH IN VARIOUS HUMAN HEMATOLOGIC MALIGNANT CELLS IN VITRO" LEUKEMIA, vol. 9, no. SUPPL. 01, October 1995, pages S118-S120, XP000602147 * the whole document * ---	1-7,9-15	
A	ZHUANG S -M ET AL: "APOPTIN, A PROTEIN DERIVED FROM CHICKEN ANEMIA VIRUS, INDUCES P53- INDEPENDENT APOPTOSIS IN HUMAN OSTEOSARCOMA CELLS" CANCER RESEARCH, vol. 55, no. 3, 1 February 1995, pages 486-489, XP000602162 * the whole document * ---	1-7,9-15	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
T	DE 196 28 894 A (HAGENMAIER HANS PAUL) 22 January 1998 * claims 1-14,16,17 * -----	1,13	

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